New Teratosphaeria species occurring on eucalypts in Australia

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Abstract

Although the first *Teratosphaeria* spp. with colletogloeopsis-like anamorphs were described outside of Australia, recently many new species have been described from Australia. In the present study, several new *Teratosphaeria* spp. were collected from infected eucalypt leaves in eastern Australia. Phylogenetic and morphological studies revealed five new taxa described here as *Teratosphaeria aurantia, T. biformis, T. foliensis, T. micromaculata* and *T. tinara.*

Keywords: *Teratosphaeria* anamorphs Colletogloeopsis-like Eucalypts Leaf disease Taxonomy Molecular phylogeny

Introduction

Teratosphaeria was established in 1912 and is typified with *T. fibrillosa* Syd and P. Syd (Crous et al. 2004a). In 2003, Taylor et al. (2003) showed that the DNA sequence of the ITS region of the type species of *Teratosphaeria* clustered within *Mycosphaerella* and thus synonymised it under *Mycosphaerella*. Later, the data based on the LSU sequence showed that *Mycosphaerella* is polyphyletic (Crous et al. 2007) contradicting earlier findings of monophyly based on ITS data (Crous et al. 2000; Goodwin et al. 2001) thus, the *Mycosphaerellaceae* was split to two families; *Mycosphaerellaceae* and *Teratosphaeriaceae*. As a result some *Mycosphaerella* spp. and anamorphs were transferred to *Teratosphaeriaceae*. Amongst them the most important are diseases of Eucalyptus caused by *M. cryptica, M. nubilosa, Kirramyces destructans (= Readeriella destructans), K. eucalypti (= R. eucalypti), K. gauchensis (= Colletogloeopsis gauchensis, = R. gauchensis*), and *K. zuluensis (= C. zuluensis = R. zuluensis).*

Although single-celled conidia of *Colletogloeopsis* species are morphologically different to multiseptate conidia of *Kirramyces* spp., Andjic et al. (2007) showed that the anamorphs of *Mycosphaerella* from eucalypt leaves and stems, currently residing in *Colletogloeopsis*, occur in a single monophyletic assemblage together with species of *Kirramyces* and thus transferred all *Colletogloeopsis* spp. to *Kirramyces*. However, Crous et al. (2007) argued that the morphological features associated with *Kirramyces* and *Colletogloeopsis* from other hosts have evolved more than once within the family *Teratosphaeriaceae* and proposed the oldest generic name *Readeriella*. As result all *Kirramyces* spp. from eucalypts were transferred to the genus *Readeriella*. Recently, in order to introduce generic names for separate lineages and not continue with dual nomenclature, several novel taxa in the *Mycosphaerellaceae* and

Teratosphaeriaceae have been proposed to try and achieve a more natural classification among the genera (Crous 2009; Crous et al. 2009b). As a result, many *Mycosphaerella* spp. and their anamorphs (including former *Kirramyces* and *Colletogloeopsis* spp.) have been transferred to the genus *Teratosphaeria*.

There have been many *Teratosphaeria* spp. described for eucalypts with colletogloeopsis-like anamorphs. The first two species described were *Colletogloeum nubilosum* = *T. cryptica* from New Zealand (Ganapathi and Corbin 1979) and *Coniothyrium ovatum* (Swart 1988) = *T. ovata* (Crous et al. 2009a). Subsequently, several species with colletogloeopsis-like anamorphs were described and included *Coniothyrium zuluense* (Wingfield et al. 1997) = *T. zuluensis* from South Africa; *Colletogloeopsis molleriana* (Crous and Wingfield 1997) = *T. molleriana* from Portugal; *Phaeophleospora toledana* (Crous et al. 2004b) = *T. toledana* from Spain; *Colletogloeopsis gauchensis* (Cortinas et al. 2006) = *T. gauchensis* from Uruguay; *C. stellenboshiana* (Crous et al. 2006) = *T. stellenboshiana* from South Africa; *Colletogloeopsis* sp. (anamorph of *T. pseudocryptica*) from New Zealand (Crous et al. 2006); and *T. verrucosa* and *T. juvenalis* from South Africa (Crous et al. 2009a), *T. hortaea* (Crous et al. 2009e) and *T. xenocryptica* from Chile (Crous et al. 2009c). Although *Teratosphaeria* spp. with colletogloeopsis-like anamorphs were first described outside Australia, recently many have been discovered in Australia such as *T. blakelyi, T. considenianae, T. dimorpha* (Summerell et al. 2006), *T. angophorae, T. corymbiae* (Andjic et al. 2007), *T. brunneotingens* (Crous et al. 2007), *T. ovata, T. veloci* (Crous et al. 2009a), *T. alboconidia, T. complicata, T. majorizuluensis, T. miniata* and *T. profusa* (Crous et al. 2009c).

During surveillance of eucalypt species trials in eastern Australia between 2005–2007, we observed leaves exhibiting symptoms associated with Mycosphaerella leaf disease (MLD). Samples were collected across several sites and preliminary microscopy examination revealed several fungi with a conidial morphology similar to that of *Teratosphaeria* spp. (colletogloeopsis-like). In the present study we describe five new *Teratosphaeria* species.

Materials and methods

Isolates

Eucalypt leaves with symptoms of MLD were collected from *Eucalyptus* and *Corymbia* spp. in southern Queensland, northern Queensland and central New South Wales. Isolates were obtained by collecting conidia exuding from single pycnidia using the tip of a sterile needle. These were transferred onto 2% Malt Extract Agar (MEA; 20 g/L Biolab malt extract, 15 g/L Biolab agar) containing Streptomycin 150 µg/ml (Sigma-Aldrich, Australia) in a single spot and allowing it to hydrate for 5 min. Under a dissecting microscope, spores were streaked across the agar using a sterile needle and single spores immediately transferred to MEA plates. Cultures were grown in the dark at 30°C for 2 weeks and then transferred to fresh MEA plates. All cultures were maintained on 2% MEA in tubes at 20°C. All isolates are maintained in the culture collection at Murdoch University (MUCC) (Table 1). Reference isolates have been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht. Herbarium specimens of new collections have been lodged in the Murdoch University herbarium (MURU). Descriptions were deposited in MycoBank.

DNA Extraction and PCR amplification

The isolates were grown on 2% MEA at 20°C for 4 weeks and the mycelium harvested and placed in a 1.5 ml sterile Eppendorf® tube. Harvested mycelium was frozen in liquid nitrogen, ground to a fine powder and genomic DNA was extracted as described previously (Andjic et al. 2007). A part of the internal transcribed spacer (ITS) region of the ribosomal DNA operon was amplified using the primers ITS-1F (5′ CTT GGT CAT TTA GAG GAA GTA A) Gardes and Bruns (1993) and ITS-4 (5′TCC TCC GCT TAT TGA TAT GC 3′) White et al. (1990). Part of the β-tubulin (βT) gene region was amplified with the primers βT2a (5′GGT AAC CAA ATC GGT GCT GCT TTC 3′) and βT2b (5′ACC CTC AGT GTA GTG ACC CTT GGC 3′) Glass and Donaldson (1995). The PCR reaction mixture, PCR conditions, the clean- up of products and sequencing were as described previously by Andjic et al. (2007).

Phylogenetic analysis

In order to compare *Teratosphaeria* isolates generated from this study with other closely related species, additional ITS sequences were obtained from GenBank. Sequence data were assembled using Sequence Navigator version 1.01 (Perkin Elmer) and aligned in Clustal X (Thompson et al. 1997). Manual adjustments were made visually by inserting gaps where necessary. All sequences derived in this study were deposited in GenBank and accession numbers are shown in Table 1.

Parsimony analysis with heuristic search was performed using PAUP (Phylogenetic Analysis Using Parsimony) (Swofford 2000) as described previously (Andjic et al. 2007). ITS trees

were rooted to *Readeriella* spp., and combined trees were rooted to *Dothistroma septospora*. Bayesian analysis was conducted on the same datasets as the one used in the distance analysis. MrModeltest v. 3.5 (Nylander 2004) was used to determine the best nucleotide substitution model. Phylogenetic analyses were performed with MrBayes v. 3.1 (Ronquist and Huelsenbeck 2003) applying a general time reversible (GTR) substitution model with gamma (G) and proportion of invariable site (I) parameters to accommodate variable rates across sites as described previously (Andjic et al. 2007). The new sequences were deposited in GenBank and the alignments and phylogenetic trees in TreeBASE (www.treebase.org).

Table 1. Isolates of new species considered in the phylogenetic study

Species	Isolate number[1]	Host	Location	Collector	GenBank accession no.	
					ß-tubulin	ITS
Teratosphaeria aurantia	MUCC668	*Eucalyptus dunnii*	QLD, Australia	G Whyte	FJ532018	EU301011
T. aurantia	MUCC669	*E. dunnii*	QLD, Australia	G Whyte	FJ532019	EU301014
T. biformis	CBS 124578	*E. globulus*	QLD, Australia	G Whyte	FJ532022	EU301002
Teratosphaeria sp.	MUCC649	*E. dunnii*	QLD, Australia	G Whyte	FJ532023	DQ240133
Teratosphaeria sp.	MUCC694	*E. dunnii*	QLD, Australia	G Whyte	FJ532024	DQ240169
T. micromaculata	CBS 124582	*E. globulus*	QLD, Australia	G Whyte	FJ532020	EU300999
T. micromaculata	MUCC648	*E. globulus*	QLD, Australia	G Whyte	FJ532021	EU301000
T. foliensis	MUCC671	*E. globulus*	NSW, Australia	S Collins	FJ532015	EU301007
T. foliensis	CBS 124581	*E. globulus*	NSW, Australia	S Collins	FJ532017	EU301006
T. tinara	MUCC665	*Corymbia* sp.	Mareeba, Australia	TI Burgess	FJ532025	EU300993
T. tinara	MUCC697	*Corymbia* sp.	Mareeba, Australia	TI Burgess	FJ532026	EU300994
T. tinara	CBS 124583	*Corymbia* sp.	Mareeba, Australia	TI Burgess	FJ532027	EU300997
T. tinara	MUCC706	*Corymbia* sp.	Mareeba, Australia	TI Burgess	FJ952514	EU300996

[1]Designation of isolates and culture collections: MUCC = Murdoch University culture collection, Australia; CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands

Culture characteristics

Plugs (2 mm diam) were cut from actively growing cultures and placed at the centre of Petri dishes (55 mm) containing one of two nutrient media. Three replicates of each isolate were grown on 2% MEA; and oatmeal agar (OMA; 30 g/L rolled oats, 20 g of Biolab agar in 1 L of distilled water) placed at 30°C in the dark. After 30 days, cultures were assessed for growth and photographed. The growth of cultures was determined by taking two measurements of colony diameter perpendicular to each other.

Morphological characterisation

Measurements of relevant taxonomic features used to distinguish between *Teratosphaeria* spp. were made. Thus, each isolate was assessed for conidial size, shape and pigmentation. Wherever possible, 30 measurements (x1000 magnification) of all taxonomically relevant structures were recorded for each species and the extremes are presented in parentheses. Colony colour was described using notations in the Munsell®Soil Color Charts (Gretag Macbeth, New Windsor, New York, revised 2000). Measurements of conidial size were obtained using the image analysis software Olysia BioReport 3.2 software imaging system. Data analyses were performed using descriptive statistics in Microsoft Excel. Conidia and conidiogenous cells were drawn using a drawing tube attached to an Olympus BH-2 microscope and following the method described by Barber and Keane (*2007*).

Results

Phylogenetic analysis

A BLASTn search was first conducted on GenBank to compare the ITS sequences of *Teratosphaeria* spp. examined in this study with those lodged in GenBank. The returned sequences were similar to *T. brunneotingens, T. considenianae, Teratosphaeria gauchensis, T. hortaea, T. multiseptata, T. majorizuluensis* and *T. zuluensis* and these and other *Teratospheria* spp. known from eucalypts were used in the phylogenetic analysis (Fig. 1, TreeBASE SN4884). The aligned ITS dataset contained 552 characters of which 201 were parsimony informative and contained significant phylogenetic signal ($P < 0.01$, $g1 = -1.25$). Parsimony analysis resulted in 107 most parsimonious trees of 739 steps ($CI = 0.48$, $RI = 0.81$). Whilst there is strong bootstrap and Bayesian support for terminal species clades and for some groups of species, there is little support for higher order clustering; *Teratosphaeria micromaculata* and *T. biformis* cluster together but are separated from each other with high bootstrap and Bayesian support. The ITS DNA sequences of *T. biformis* (CBS 124578) and *Teratosphaeria* sp. (MUCC648 and MUCC694) were identical. However, phylogeny based on combined ITS and βT gene regions did not support this association (Fig. 2, TreeBASE SN4443). Furthermore, *T. biformis* and the undescribed *Teratosphaeria* sp. have different cultural characteristics (colour, appearance, growth rate and sporulation). The *Teratopshearia* sp. was not described, as on the original leaf material it is found in association with other similar species and it does not sporulate in culture. Thus a direct link between the morphological observations on the leaf material and in culture could not be made. *Teratosphaeria tinara* resides in a strongly supported terminal clade, clustering with *T. multiseptata. Teratosphaeria aurantia* resides alone in a strongly supported terminal clade. *Teratosphaeria foliensis* grouped in a clade containing *T. considenianae, T. stellenboschiana,*

T. gauchensis, *T. majorizuluensis* and *T. zuluensis*. This clade could not be clearly resolved on ITS data alone, but in the combined analysis with βT each species was clearly resolved with bootstrap values of 100% and posterior probabilities of 1.00 (Fig. 2, TreeBASE SN4443).

Taxonomy

Morphological comparison of *Teratosphaeria* spp. with colletogloeopsis-like anamorphs found on eucalypts are presented in Table 2.

Teratosphaeria aurantia Whyte & Andjic, **sp. nov.**

MycoBank MB 514050 (Figs. 3, 25–28)

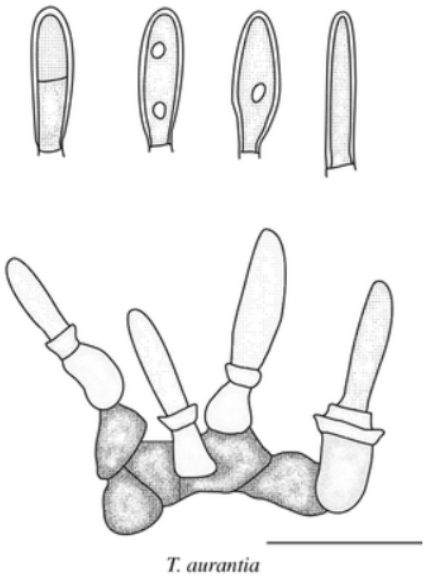


Fig. 3 Conidia and conidiogenous cell of Teratosphaeria aurantia. Scale bar = 10 μm

Etymology: named after the golden yellow stain of the agar.

Simile ut *T. pseudocryptica*, sed differret conidiis tenuioris et discoloratione aureo-flava agaris.

Leaf spots epiphyllous and hypophyllous, extending through leaf lamina, pale brown, conspicuously circular 0.5–5 mm diam with corky-brown margins. *Mycelium* immersed in host tissue, septate, branched, melanised. *Conidiophores* reduced to conidiogenous cells. *Conidiomata* pycnidial, embeded, sub-epidermal, separate, globose, wall consisting of 4–5 layers of dark brown *textura angularis*. *Conidiogenous cells* subcylindrical to doliiform, subhyaline to medium brown, smooth, proliferating percurrently with 1–2 annulations, formed from the inner cells of the pycnidial wall, 5.5 × 4.0 μm. *Conidia* ellipsoidal, 0–1 septate, subhyaline to medium brown, smooth, guttulate, gradually tapering toward apex, truncate to subtruncate at base with marginal frill, (9.5–)11–14(–16.0) × (2.5–)2.5–3.5(–4.0) (mean = 12.5 × 3.0 μm).

Cultural characteristics Colonies on MEA reaching a diam 4 × 5 mm after 1 month at 30°C, globular aggregating or separate masses with white to cream 2Y 8/3 short aerial hyphae on the surface; dark brown 10YR 3/3 in reverse. On OMA colonies reaching 7 × 8 mm diam, after 1 month, globular aggregating or separate masses with white to cream 2Y 8/3 short aerial hyphae on surface; dark brown 10YR 3/3 in reverse.

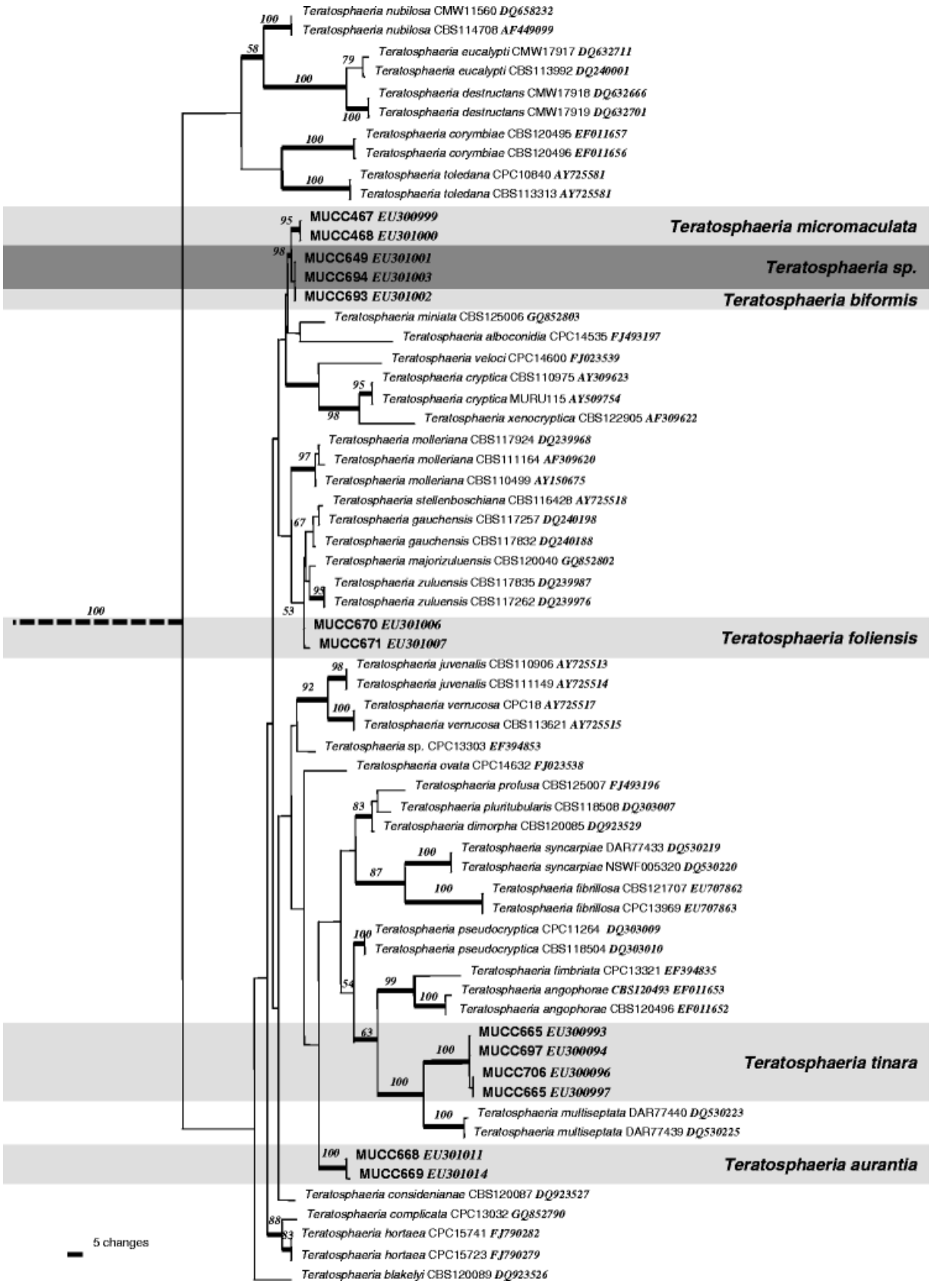

Teratosphaeria nubilosa CMW11560 DQ658232
Teratosphaeria nubilosa CBS114708 AF449099
Teratosphaeria eucalypti CMW17917 DQ632711
Teratosphaeria eucalypti CBS113992 DQ240001
Teratosphaeria destructans CMW17918 DQ632666
Teratosphaeria destructans CMW17919 DQ632701
Teratosphaeria corymbiae CBS120495 EF011657
Teratosphaeria corymbiae CBS120496 EF011656
Teratosphaeria toledana CPC10840 AY725581
Teratosphaeria toledana CBS113313 AY725581
MUCC467 EU300999
MUCC468 EU301000
Teratosphaeria micromaculata
MUCC649 EU301001
MUCC694 EU301003
Teratosphaeria sp.
MUCC693 EU301002
Teratosphaeria biformis
Teratosphaeria miniata CBS125006 GQ852803
Teratosphaeria alboconidia CPC14535 FJ493197
Teratosphaeria veloci CPC14600 FJ023539
Teratosphaeria cryptica CBS110975 AY309623
Teratosphaeria cryptica MURU115 AY509754
Teratosphaeria xenocryptica CBS122905 AF309622
Teratosphaeria molleriana CBS117924 DQ239968
Teratosphaeria molleriana CBS111164 AF309620
Teratosphaeria molleriana CBS110499 AY150675
Teratosphaeria stellenboschiana CBS116428 AY725518
Teratosphaeria gauchensis CBS117257 DQ240198
Teratosphaeria gauchensis CBS117832 DQ240188
Teratosphaeria majorizuluensis CBS120040 GQ852802
Teratosphaeria zuluensis CBS117835 DQ239987
Teratosphaeria zuluensis CBS117262 DQ239976
MUCC670 EU301006
MUCC671 EU301007
Teratosphaeria foliensis
Teratosphaeria juvenalis CBS110906 AY725513
Teratosphaeria juvenalis CBS111149 AY725514
Teratosphaeria verrucosa CPC18 AY725517
Teratosphaeria verrucosa CBS113621 AY725515
Teratosphaeria sp. CPC13303 EF394853
Teratosphaeria ovata CPC14632 FJ023538
Teratosphaeria profusa CBS125007 FJ493196
Teratosphaeria pluritubularis CBS118508 DQ303007
Teratosphaeria dimorpha CBS120085 DQ923529
Teratosphaeria syncarpiae DAR77433 DQ530219
Teratosphaeria syncarpiae NSWF005320 DQ530220
Teratosphaeria fibrillosa CBS121707 EU707862
Teratosphaeria fibrillosa CPC13969 EU707863
Teratosphaeria pseudocryptica CPC11264 DQ303009
Teratosphaeria pseudocryptica CBS118504 DQ303010
Teratosphaeria fimbriata CPC13321 EF394835
Teratosphaeria angophorae CBS120493 EF011653
Teratosphaeria angophorae CBS120496 EF011652
MUCC665 EU300993
MUCC697 EU300094
MUCC706 EU300096
MUCC665 EU300997
Teratosphaeria tinara
Teratosphaeria multiseptata DAR77440 DQ530223
Teratosphaeria multiseptata DAR77439 DQ530225
MUCC668 EU301011
MUCC669 EU301014
Teratosphaeria aurantia
Teratosphaeria considenianae CBS120087 DQ923527
Teratosphaeria complicata CPC13032 GQ852790
Teratosphaeria hortaea CPC15741 FJ790282
Teratosphaeria hortaea CPC15723 FJ790279
Teratosphaeria blakelyi CBS120089 DQ923526
5 changes

Fig. 1 One of the 107 most parsimonious trees of 739 steps resulting from analysis of the ITS dataset. The complete tree can be viewed at TreeBASE (SN4884). Numbers in italics represent bootstrap support for the nodes. Thickened branches indicate a posterior probability based on Bayesian analysis of greater than 0.80. The new Teratosphaeria species described in this study are highlighted

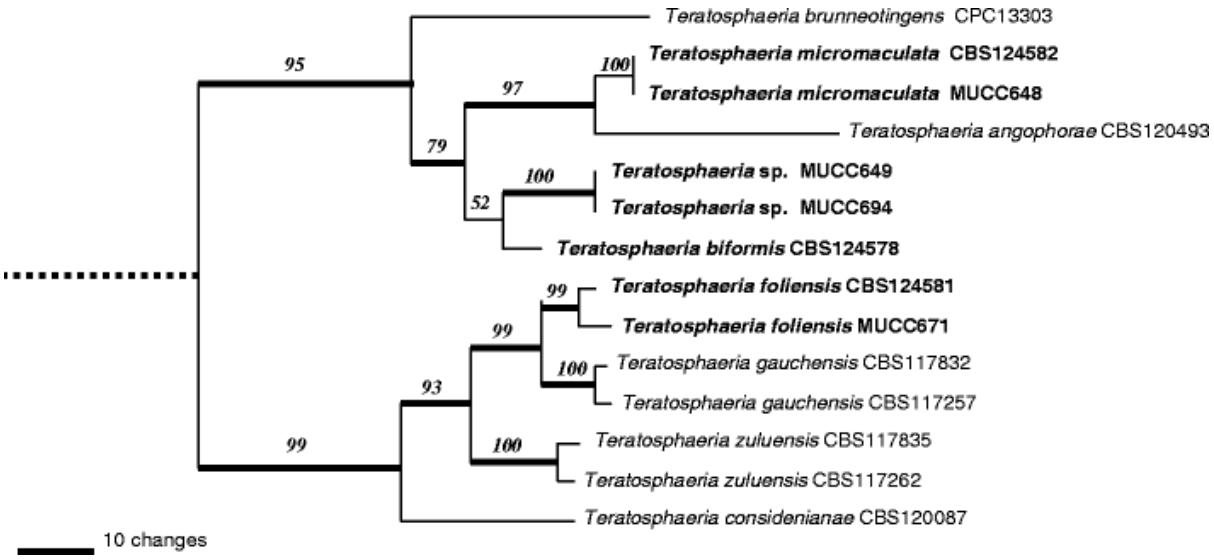


Fig. 2 Part of one of 37 most parsimonious trees of 1270 steps resulting from the combined ITS and β-tubulin dataset illustrating the strong support for the separation of T. foliensis from T. zuluensis and T. gauchensis and T. biformis from an undescribed Teratosphaeria sp. Numbers in italics represent bootstrap support for the nodes. Thickened branches indicate a posterior probability based on Bayesian analysis of greater than 0.80. The complete analysis and resultant trees are available from TreeBASE SN4439

Material examined ***Australia****, Queensland, Rosedale, on leaves of Eucalyptus grandis, G. Whyte 2007, holotype MURU440, culture ex-type MUCC668, CBS125243. Additional specimens*: **Australia**, Queensland, Rosedale, on leaves of *Eucalyptus dunnii*, G. Whyte 2007, (MURU439) (culture ex-type MUCC669).

Notes *Teratosphaeria aurantia* is phylogenetically and morphologically similar to the anamorph of *T. pseudocryptica* (12–14 × 4 μm). However, it can be distinguished from the latter species by the golden-yellow pigment in the agar, and slightly thinner conidia (11–14 × 2.5–3.5 μm). In addition, the lesions with which *T. aurantia* is associated are distinct in appearance from other described *Teratosphaeria* species with distinctly circular and raised margins with an aggregation of fruiting structures in the centre (Fig. 9).

Teratosphaeria biformis Whyte &Andjic, **sp. nov**.

MycoBank MB 514051 (Figs. 4, 12–16)

Etymology: named after its ability to produce conidia both as a coelomycete and hyphomycete on the leaf and as a hyphomycete on agar.

Simile ut *Teratosphaeria hortaea*, quaternus cellulae conidiogenae producentur de cellulis hypharum, sed conidiae differrent in forma et magnitudini et absentia septarum.

Leaf spots epiphyllous and hypophyllous, pale brown, conspicuously circular, 0.5–5 mm diam, extending through leaf lamina. *Mycelium* immersed in host tissue, septate, branching, melanised. *Conidiophores* absent. *Conidiomata* pycnidial, dark brown, amphigenous, aggregated, globose. Conidiogenous cells globular, brown, smooth. *Conidia* holoblastic, subhyaline but becoming melanised when mature, aseptate, ovoid, thick-walled, truncate at base with a minute marginal frill, (6.0–)7–10(–11.0) × (2.5–)3–4(–4.0) (mean = 8.5 × 3.5 µm).

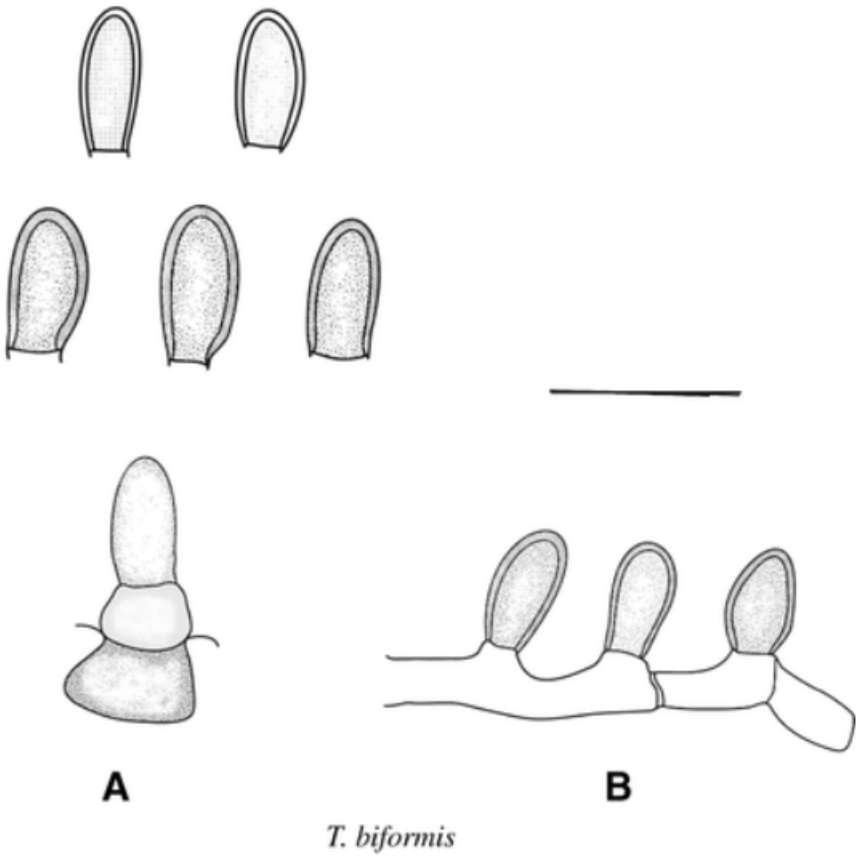


Fig. 4 A) Conidia and conidiogenous cell of Teratosphaeria biformis in vivo; B) Conidiogenesis from hyphae in vitro. Scale bar = 10 µm

Cultural characteristics Colonies on MEA reaching diam 45 × 45 mm after 1 month at 30°C, regular with smooth margins, pale gray 2.5Y 7/3 with pale yellowish brown mycelium 2.5Y 6/3 on surface; reverse olive-brown 2.5Y 4/4 with white margins. On OMA colonies reaching 25 × 25 mm diam, regular with smooth margins distributed in three concentric zones of different colours, out zone pale grey 2.5Y 7/1, middle pale brown-grey 6/2 and inner zone pale grey 7/1 on top; reverse side is the same as surface.

Material examined **Australia**, Queensland, Rosedale, on leaves of Eucalyptus globulus, G. Whyte, 2007, MURU438, culture ex-type MUCC693, CBS124578.

Notes *T. biformis* is phylogenetically closest to *T. micromaculata* from which it differs by slightly longer and wider conidia (7–10 × 3–4 μm), *T. micromaculata* (5–7 × 2–3 μm). *T. biformis* is morphologically closest to *T. hortaea* as it produces conidiogenous cells randomly on hyphal cells but it can easily be distinguished by its conidial shape, size and septation (*T. biformis* = conidia ovoid, 8.5–10.5 × 3.5–4.5 μm *in vitro*, aseptate, *T. hortaea* = conidia ellipsoid, 5–6 × 2.5 μm *in vitro*, 0–1 septa).

Teratosphaeria foliensis Andjic & S. Jackson, **sp. nov.**

MycoBank MB 514053 (Figs. 5, 17–20)

Etymology. Name refers to leaf, follium = leaf, ensis = refers to its close phylogenetic relationship to *T. gauchensis, T. majorizuluensis* and *T. zuluensis*.

Simile ut *Teratosphaeria blakelyi*, sed differret conidiis late ellipsoideis.

Leaf spots raised, pale brown, circular up to 9 mm diam; border medium to dark brown, raised with a purple margin. *Conidiomata* semi-immersed, pycnidial, aggregated in circle, globose, erupment, black; wall of 2–3 layers of dark brown *textura angularis*. *Conidiogenous cells* subhylaline to pale brown, doliiform to subcylindrical, annellidic, proliferating percurrently, 4–8 × 2–4 μm (mean = 6 × 3 μm). *Conidia* solitary, hyaline to subhyline, guttulate, aseptate, smooth, ellipsoidal to obovoid, base truncate to subtruncate with marginal frill, apex obtuse, (8–)8.5–11(–12.5) × (2.7–)3–3.5(–5) (mean = 10.5 × 3.5 μm), culture sterile.

Cultural characteristics Colonies on MEA: reaching diam 35 × 35 mm after 1 month at 30°C, margins regular, surface olive-grey 5Y 5/2 with moderate aerial mycelium; reverse dark olive-grey 5Y 3/2. On OMA colonies reaching 50 × 50 mm in diam, margins regular,

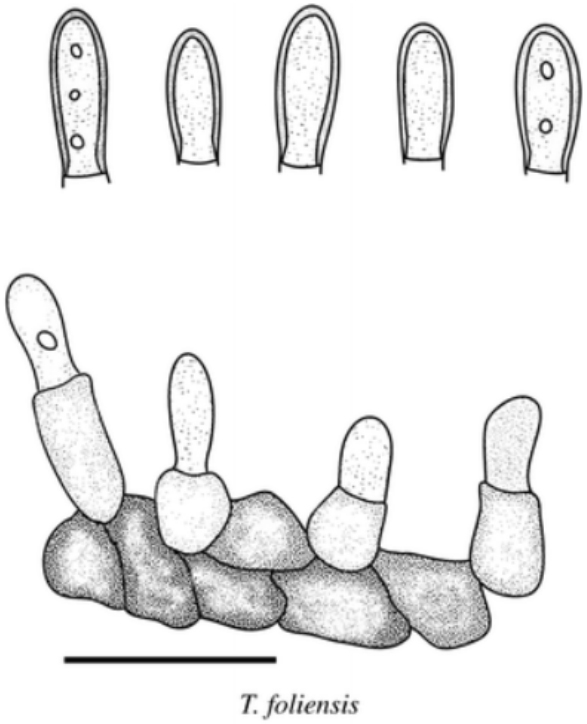


Fig. 5 Conidia and conidiogenous cell of Teratosphaeria foliensis. Scale bar = 10 μm

surface olive-brown 2.5Y 4/2 with patches of pale olive-brown and grey aerial mycelium; reverse grayish brown 2.5Y 5/2.

Material examined **Australia**, New South Wales, Commins plantation, 146°9′56″ 34°35′1″, on leaves of *Eucalyptus globulus*, May 2005, Sally Collins, S. Jackson, holotype MURU442, culture ex-type MUCC670, CBS124581. *Additional specimens*: **Australia**, New South Wales, Graham plantation, on leaves of *Eucalyptus globulus*, May 2005, Sally Collins, S. Jackson, culture ex-type MUCC696.

Notes *T. foliensis* is phylogenetically closely related to *T. zuluensis* and *T. majorizuliensis*, but it can be distinguished from it by its longer conidia (*T. foliensis* = 8.5–11 × 3–3.5 μm, *T. zuluensis* = 4.5–5 × 2–2.5 μm and *T. majorizuluensis* = 5–6 × 2 μm) and pigmentation (*T. foliensis* = hyaline to subhyline, and *T. zuluensis* subhyline to pale brown, *T. majorizuluensis* = brown.) Morphologically, *T. foliensis* (conidial dimensions) is closest to T. *blakelyi* (9–10 × 3 μm), but can be distinguished from *T. blakelyi* by the shape of conidia; *T. blakelyi* has been characterised by having narrowly ellipsoidal conidia while *T. foliensis* has widely ellipsoidal conidia.

Table 2

Morphological features of conidia of *Teratosphaeria* species from eucalypts with colletogloeopsis-like anamorphs recorded in the published literature and in the present study; *in vivo* = herbarium specimens, *in vitro* = isolates from culture, n/a = not applicable (the isolates were sterile in culture or were not available). Species are presented in order of conidia size from smallest to largest

Species	Herbarium number	Conidia				Conidiogenous cell
		size *in vivo* (µm)	size *in vitro* (µm)	pigmentation	septation	size *in vivo* (µm)
T. zuluensis (Cortinas et al. 2006)	IMI 370886	4.5–5×2–2.5	n/a	Subhyaline to pale brown	0–1	4–8×2–3.5
T. hortaea (Crous et al. 2009d)	CBS-H-20194	n/a	5–6×2.5	Pale to medium brown	0–1	n/a
T. alboconidia (Crous et al. 2009c)	CBS 1250004	n/a	5–6×2.5–3	Pale brown to brown	0	n/a
T. majorizuluensis (Crous et al. 2009c)	CBS H-19773	5–6×2	6–9×2.5–4	Brown	0	5–7×2–3
T. micromaculata Present study	MURU 437	5–7×2–3	7–8×2.2	Hyaline to pale brown	0	5×4
T. gauchensis (Cortinas et al. 2006)	CBS 19722	5–6×2.5	n/a	Medium brown	0	n/a
T. miniata (Crous et al. 2009c)	CBS H-20269	6–7×3	10×5	Brown	0–1	4–8×2.5–3.5
T. brunneotingens (Crous et al. 2007)	CBS-H 19838	6–7×2–3	n/a	Brown	0–1	5–7×3–5
T. tinara Present study	MURU 445	6–7.5×3–3.5	n/a	Subhyaline to pale brown	0	5–6×3–3.5
T. considenianae (Summerell et al. 2006)	CBS H-19744	7–9×3	n/a	Medium brown	0	3–6×4–5
T. stellenboschiana (Crous et al. 2006)	CBS H-19688	7–9×3.5	n/a	Medium brown	0	3–6×3–4
T. verrucosa (Crous et al. 2009a)	CBS H-20183	7–9×5	8–10×5	Hyaline to brown	0	5–10×4–6
T. ovata (Crous et al. 2009a)	DAR 49461	7–10×3–3.5	n/a	Brown	0	3–6×4–6
T. xenocryptica (Crous et al. 2009c)	CBS 122905	n/a	7–15×2.5–3.5	Pale to medium brown	0	5–15×3–5
T. profusa (Crous et al. 2009a)	CBS 125007	n/a	8–10×3	Brown	0–1	n/a
T. biformis Present study	MURU 438	7–10×3–4	8.5–10.5×3.5–4.5	Pale brown to brown	0	n/a
T. veloci (Crous et al. 2009a)	CBS H-20182	n/a	8–10×3	Brown	0	n/a
T. foliensis Present study	MURU 442	8.5–11×3–3.5	n/a	Hyaline to pale brown	0	4–8×2–4
T. blakelyi (Summerell et al. 2006)	CBS H-19743	9–10×3	n/a	Pale brown	0	5–7×3–4
T. dimorpha (Summerell et al. 2006)	CBS H-19739	9–11×4	n/a	Medium brown	0	7–15×3–5
T. molleriana (Crous and Wingfield 1997)	PREM 54395	9–12×3–3.5	n/a	Medium brown	0	4–20×3.5–5
T. angophorae (Andjic et al. 2007)	DAR 77452	9–15×2.5–4	10.5–22.5×3–4.5	Sub-hyaline to pale brown	0–3	6.5–12×2.5–4
T. toledana (Crous et al. 2004b)	CBS 59896	10–12×3–3.5	n/a	Pale brown	0	6–10×3–4
T. nubilosum (=*T. cryptica*) (Crous and Wingfield 1997)	PDD 37677	10–15×4–5	n/a	Medium brown	0	5–10×4–7
T. juvenalis (Crous et al. 2009a)	CBS H-20180	11–13×5	n/a	Hyaline to brown	0	3–12×5–6
T. aurantia Present study	MURU 440	11–14×2.5–3.5	n/a	Pale brown	0–1(2)	5.5×4
T. pseudocryptica (Crous et al. 2006)	CBS H-19693	12–14×4	n/a	Medium brown	0	5–15×3–5
T. corymbiae (Andjic et al. 2007)	DAR 77445	17–23×3.5–5	16.5–22×2.5–3.5	Pale brown	0	6–13

[1] Designation of isolates and herbarium collections: CBS-H = Centraalbureau voor Schimmelcultures Utrecht Netherlands; PDD = New Zealand Fungal Herbarium, Auckland, New Zealand, PREM = National collection of fungi, Pretoria, South Africa, MURU = Murdoch University herbarium collection, Perth; Australia; DAR = New South Wales, Plant Pathology Herbarium, Australia; NSWF = Forest Research Culture Collection, New South Wales, Australia; IMI = International Mycological Institute, CABI Bioscience, Egham, UK. New species are labeled in bold.

Teratosphaeria micromaculata Whyte & Andjic, **sp. nov.**

MycoBank MB514054 (Figs. 6, 21–24)

Etymology: named after its association with relatively small lesion spots.

Simile ut *Teratosphaeria gauchensis*, sed differret absentia conidiophororum.

Leaf spots epiphyllous and hypophyllous, dark brown circular lesion 0.5–2 mm diam, with a raised purple margin followed by a pale brown margin, extending through leaf lamina. *Mycelium* immersed in host tissue, septate, branching, melanised. *Conidiophores* absent. *Conidiomata* acervular, globular, superficial with very little of the epidermis remaining intact. *Conidiogenous cells* annelidic, globular to dolliform, medium brown, smooth, proliferating percurrently (4.0–) 5 (–5.6) × (4.0–) 4 (–4.8). *Conidia* ellipsoidal to ovoid, aseptate, thick-walled, hyaline when produced but becoming melanised, truncate to subtruncate at base with marginal frill, (5.0–) 5–7 (–7.5) × (2.0–)2–3(–3.5) (mean = 6.0 × 2.5 μm).

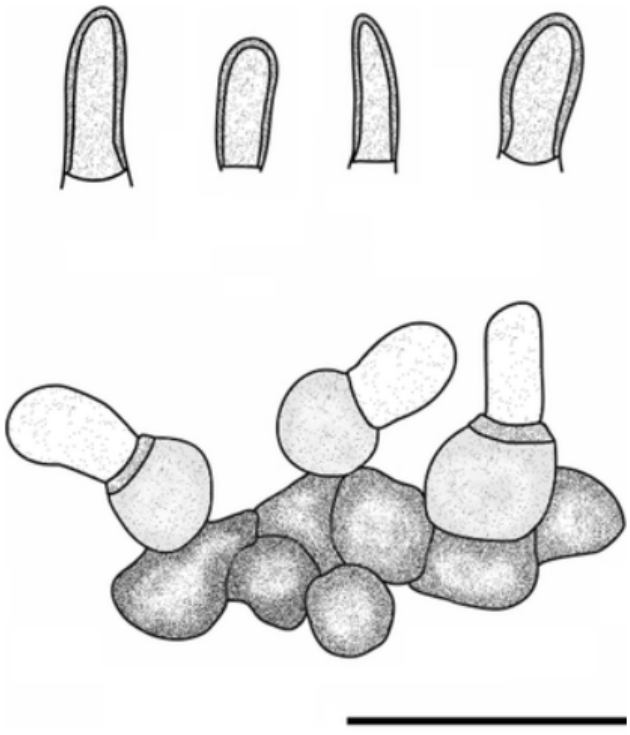


Fig. 6 Conidia and conidiogenous cell of Teratosphaeria micromaculata. Scale bar = 10 μm

Cultural characteristics Colonies on MEA reaching diam. 8 × 12 mm after 1 month at 30°C, irregular with smooth margins, dark olive-brown 2.5Y 3/3 with darker margins, pale olive-brown 2.5Y 5/4 aerial hyphae. On OMA colonies reaching 12 × 15 mm, pale olive-brown 2.5Y 5/4 mixed with pale cream hyphae, rough, lightly furred.

Material examined **Australia**, Queensland, Boonah, on leaves of *Eucalyptus globulus*, G. Whyte, 2007, holotype MURU437, culture ex-type MUCC647, CBS124582. *Additional specimens*: **Australia**, Queensland, Boonah, on leaves of *Eucalyptus globulus*, G. Whyte, 2007, (culture ex-type MUCC648).

Notes *T. micromaculata* is phylogenetically closest to *T. biformis* but differs by slightly smaller conidia (5–7 × 2–3 μm) than *T. biformis* (7–10 × 3–4 μm). Morphologically, *T. micromaculata* is somewhat similar in conidial shape and size to *T. gauchensis* (5–6 × 2.5 μm). However, it can be easily distinguished from *T. gauchensis* by its lack of conidiophores as it produces conidia directly from conidigenous cells.

Teratosphaeria tinara Andjic & T.I. Burgess, **sp. nov.**

MycoBank MB 514056 (Figs. 25–28)

Etymology: refers to the Lake Tinaroo where the fungus was collected.

T. tinara differet anamorphis aliis generis *Teratosphaeria* formis *variabilibus* conidiarum: late ellipsoidea ad obovoidea.

Leaf spots amphigenous, irregular blotches, 1–4 mm diam, pale, brown with purple border. *Conidiomata* semi-immersed, pycnidial, single or aggregated, globose up to 112 μm diam, erupment, dark brown. *Conidiophores* absent. *Conidiogenous cells* subhyaline, doliiform to subcylindrical, smooth, proliferating percurrently with irregular annulations, 5–6 × 3–3.5 (mean = 5 × 3.3 μm). *Conidia* subhyaline becoming brown when mature, aseptate, guttulate, thick-walled, obovoid to broadly ellipsoidal, base truncate to subtruncate with minute marginal frill, apex obtuse, (3.5–)6.0–7.5(–9.5) × (1.8–)3.0–3.5(4.4–) (mean = 6.5 × 3.1 μm), culture sterile.

Cultural characteristics Colonies on MEA: reaching diam 13 × 12 mm after 1 month at 30°C, margins irregular, surface black 7.5YR 2.5/1, aerial mycelium grey; reverse very dark brown 7.5YR 2.5/3. On OMA colonies reaching 12–10 mm diam., surface very dark grayish

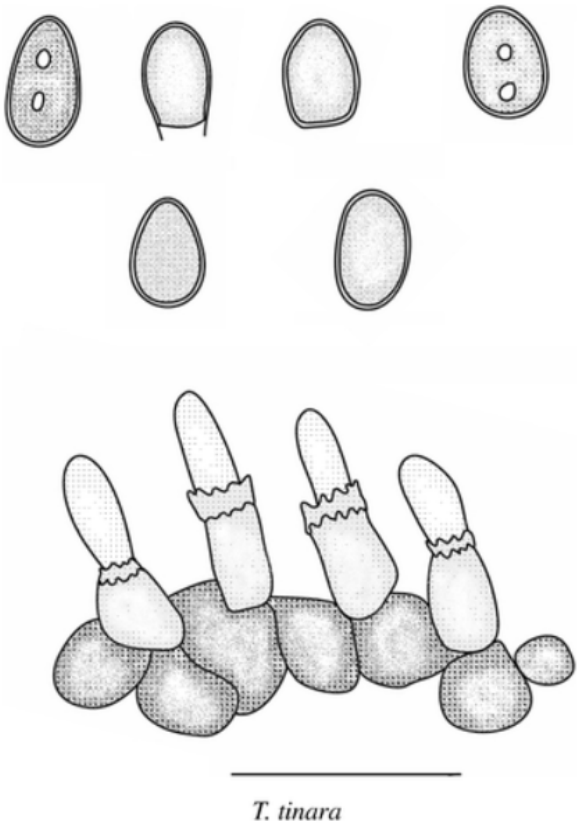
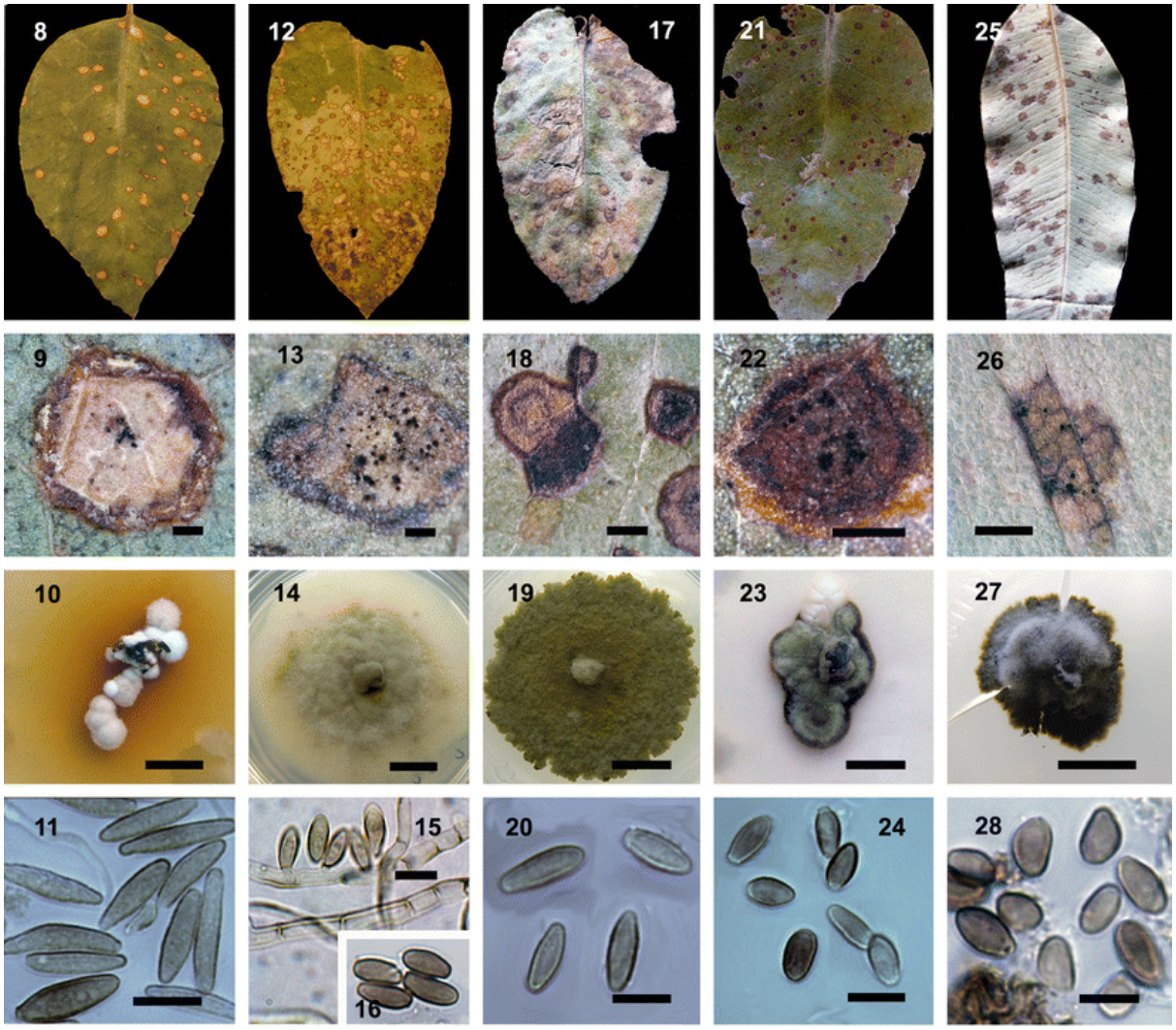


Fig. 7 Conidia and conidiogenous cell of Teratosphaeria tinara. Scale bar = 10 µm

brown 2.5Y 5/2 with pale grey areal mycelium 2.5Y 7/1, margins irregular; reverse olive-brown 2.5Y 4/4.

Material examined Australia, northern Queensland, Mareeba, Lake Tinaroo, on leaves of *Corymbia* sp., August 2006, T.I. Burgess, holotype MURU445, culture ex-type MUCC666 = CBS124583). *Additional specimens*; Australia, northern Queensland, Mareeba, on leaves of *Corymbia* sp., August 2006, T.I. Burgess, MURU444, culture ex-type MUCC665.

Notes Based on ITS DNA sequence, *T. tinara* is closely related to *T. multiseptata* but can not be compared with it directly as the anamorph of *T. multiseptata* has not been seen. Most anamorphs of *Teratosphaeria* spp. (colletgloeopsis-like) have similar conidia shape ranging from ellipsoidal to subcylindrical and fusiform shape. However, *T. tinara* differs by producing both ellipsoidal and obovoid conidia shape.



Figs. 8–28

Teratosphaeria aurantia: 8. Leaf symptoms; 9. Lesion with pycnidia aggregating in the centre, scale bar = 1 mm; 10. Colony on MEA, scale bar = 10 mm; 11. Conidia *in vivo*, scale bar = 10 µm. ***Teratosphaeria biformis***: 12. Leaf symptoms; 13. Lesion with pycnidia, scale bar = 1 mm; 14. Colony on MEA, scale bar = 10 mm; 15. Sporulation on aerial hyphae *in vitro*, scale bar = 10 µm; 16. Conidia *in vivo*, scale bar = 10 µm. ***Teratosphaeria foliensis***: 17. Leaf symptoms; 18. Lesion with pycnidia aggregating in circle, scale bar = 1 mm; 19. Colony on MEA, scale bar = 10 mm; 20. Conidia *in vivo*, scale bar = 10 µm. ***Teratosphaeria micromaculata***: 21. Leaf symptoms; 22. Lesion with pycnidia, scale bar = 1 mm; 23. Colony on MEA, scale bar = 10 mm; 24. Conidia *in vivo*, scale bar = 10 µm. ***Teratosphaeria tinara***: 25. Leaf symptoms; 26. Lesion with pycnidia, scale bar = 1 mm; 27. Colony on MEA, scale bar = 10 mm; 28. Conidia *in vivo*, scale bar = 10 µm

Discussion

Teratosphaeria spp. and their anamorphs include some of the most important pathogens of eucalypts (Crous 2009). Many of them have been moved around the world through the establishment of eucalypt plantations. In recent years numerous new *Teratosphaeria* spp. have been described (Crous et al. 2006, 2007, 2009a, b, c, d; Summerell et al. 2006; Andjic et al. 2007) including the five new species in this study. Many species of *Teratosphaeeria* are morphologically indistinguishable and their taxonomy relies heavily on DNA sequence comparison (Cortinas et al. 2006; Hunter et al. 2006; Andjic et al. 2007; Crous 2009, Crous et al. 2009b).

T. aurantia and *T. biformis* were isolated from *E. dunnii* and *E. grandis* plantations in southern Queensland but the incidence and severity they pose to eucalypt plantations is uncertain. *T. micromaculata* was found on *E. globulus* in southern Queensland with incidence and severity ranked as low. *T. foliensis* was found in a plantation on *Eucalyptus globulus* in New South Wales. Although, very closely related to the serious pathogen *T. zuluensis* and *T. gauchensis* which cause a stem canker disease, *T. foliensis* was symptomatic rather than damaging. *T. tinara* was isolated from native *Corymbia* sp. from north Queensland and it is probably native to the region.

To date, there is sequence data and type cultures for 28 *Teratosphaeria* spp. described from eucalypts for which a colletogloeopsis-like anamorph is known. Conidia range in size from the smallest *T. zuluensis* (4.5–5 × 2–2.5 μm) to *T. corymbiae* (17–23 × 3.5–5 μm), pigmentation and septation also varies, but generally condia are aseptate. With the exception of stem canker pathogens *T. zuluensis* and *T. gauchensis*, the rest of these species produce lesions on leaves with various symptoms. All except *T. gauchensis, T. juvenalis, T. pseudocryptica, T. zuluensis, T. stellenbochiana, T. xenocryptica* and *T. verrucosa* have been reported in Australia.

The *Teratopshaeria* spp. with colletogloeopsis-like anamorphs are related to the most damaging leaf disease species (kirramyces-like anamorphs) found on *Eucalyptus*; *T. destructans, T. eucalypti,* and *T. viscidus*. Unlike the latter species, no species with a colletogloeopsis-like anamorphs has been found to cause major leaf diseases in Australia with the exception of *T. cryptica (=T. nubilosum*). The expansion of eucalypt plantation forestry into sub-tropics of Australia has led to the discovery of many new *Teratopshaeria* spp. and they now seem to be the dominant genus on sub-tropical eucalypts. Currently, the species described in this study are not causing any significant damage to Australian eucalypt plantations and incidence and threat they may pose to forest industry is unknown.

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